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Use of Nitric Oxide Donors for the Treatment of Obstetric and Gynecological Pathologies in Cows

Zinaida Artyushina[1], Sergey Fedotov[1], Vladimir Serezhenkov[2], Nikita Sidnev[1], Nikolaj Tkachev[2], Marina Kuznetsova[3] and Zhora Muradyan[3]

[1]Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Russia; [2] NN Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Russia; [3]Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Skryabin, Russia.
*Corresponding author's e-mail: zinaida.artyushina@mymail.academy

Intrauterine infections in cattle are a major contributor to reduced reproductive efficiency and infertility, leading to significant economic losses. Conventional treatments often provide limited long-term results, especially in cases of chronic endometritis. This study evaluates the therapeutic potential of nitric oxide (NO) donors, specifically dinitrosyl iron complexes (DNICs) with thiol ligands, in treating bovine uterine inflammatory disease. A multistage approach was employed: in vitro assays tested the bactericidal activity of DNIC-glutathione against Staphylococcus aureus, while an in vivo rat model of surgically induced endometriosis served as a mechanistic and dose-finding stage. These preclinical data informed the selection of veterinary dosing and formulations. A pilot veterinary study was conducted on four cows with chronic endometritis unresponsive to standard treatments. Clinical signs included persistent low-grade fever (+0.5-1.0 °C), flaccid enlarged uterus, petechial vaginal mucosa, and purulent or hemorrhagic discharge. Diagnosis was confirmed through clinical, cytological, and bacteriological methods, revealing mixed microbial flora (Escherichia coli, Pseudomonas aeruginosa, cocci, and fungi). Animals received intrauterine DNIC-glutathione (180 µg/kg) once daily for five days. Post-treatment evaluation showed a 4-5 fold decrease in microbial load, with elimination of P. aeruginosa in two cows and E. coli in three. Fungal and anaerobic growth ceased, and uterine tissue normalized macroscopically. These findings suggest that DNIC-based NO donors offer both antimicrobial and anti-inflammatory benefits for treating bovine endometritis. The integration of laboratory and field models supports a defined translational pathway and justifies further controlled trials to assess efficacy, reproductive outcomes, and long-term safety in veterinary applications.

Keywords: Nitric oxide donor, dinitrosyl iron complexes, DNIC-glutathione, bovine endometritis, intrauterine therapy, veterinary gynecology, antimicrobial therapy, pilot clinical trial.

INTRODUCTION

Conventional treatments for bovine uterine infections like endometritis often show limited long-term efficacy, leading to significant reproductive and economic losses. This study hopes to investigate the potential of nitric oxide (NO) donors, specifically dinitrosyl iron complexes (DNICs), as a more effective therapeutic strategy. In the early postpartum period, dairy cows face challenges such as birth canal damage, placental detachment, systemic inflammation and metabolic stress, immunological suppression, and uterine microbiome alterations. Inability to overcome one or more of these obstacles can lead to an increase in various types of reproductive tract inflammatory illness, which affects up to half of postpartum dairy cows. Dairy cows can develop metritis within 21 days of giving birth (although more commonly between 3 and 10 days). Metritis is defined histologically as inflammation of all uterine layers (endometrium, myometrium, and perimetrium) and is clinically distinguished by fetid uterine discharge, which can be reddish-brown and watery or purulent and viscous, and is frequently associated with an enlarged, flaccid uterus (Pascottini et al., 2023). The clinical significance of endometritis cannot be overlooked. Husnain et al.

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(2023) reported that induced endometritis reduced yields of milk, pregnancy per insemination, protein, lactose, and dry matter intake. Due to its detrimental effects on reproductive performance and the ensuing financial losses for farmers, endometritis in cows is clinically significant. It results in higher rates of culling, longer intervals between calving and pregnancy, and decreased fertility. The multifactorial nature of the illness, which includes intricate microbial infections and immune reactions that can harm the uterus and postpone pregnancy, makes diagnosis and treatment difficult (Kasimanickam et al., 2025; Paiano et al., 2022). Boni and Gualandi (2022) highlighted in their study that there is a relationship between endometritis and oxidative stress. They reported that in cattle, NO has been found in the mammalian uterus with endometritis, causing uterine relaxation; it is controlled by steroid hormones but may also alter the structure or action of these hormones. Furthermore, resistant mares had a reasonably consistent level of total intrauterine NO 24 hours after breeding, but susceptible mares had a large rise in total NO compared to resistant mares at 6 and 12 hours. Additionally, in mares, endometritis has been linked to oxidative processes. Arabian mares with endometritis had considerably higher plasma MDA levels and reduced glutathione peroxidase (GPx) activity in erythrocytes.

Studies have displayed that the standard endometritis treatments in cows include antibiotics and prostaglandin, but these treatment plans are faced with prevalent limitations such as the presence of a knowledge gap, especially in rural and sub-Saharan regions. The possibility of antimicrobial resistance is also a limitation to using antibiotics in treating endometritis. Kasimanickam et al. (2025) also explained that for postpartum endometritis cases, biofilms can form on the uterine lining, resulting in a chronic source of infection that is very difficult to eliminate. This structural protection reduces antibiotic penetration and allows germs to survive therapy, which contributes to chronic or recurring infections. Furthermore, bacteria in biofilms undergo genotypic and phenotypic changes, such as changed gene expression, which promotes virulence, inhibits immune detection, and increases resistance to conventional therapy. There is also the risk of antibiotic cross-transmission to humans through dairy and meat products (Nyabinwa et al., 2020; Huerta-Saquero et al., 2025). In addition to alternative therapies like nanoparticles and uterine lavage, there have been recent studies on the potential of nitric oxide (NO) donors as a novel therapy for detecting and modifying the inflammatory and immune response. Although elevated NO levels are linked to inflammation, modifying this system with NO donors may lessen it and help with therapy (Artyushina et al., 2024; Oladejo et al., 2021).

Currently several preparations are widely used in veterinary medicine, the activity of which is reasonably associated with their ability to release nitric oxide (NO) in vivo. Metronizadol, which is widely used in veterinary and medical practice, forms the dinitrosyl iron complex (DNIC) (Mason and Josephy, 1985), as well as nitrobenzofuroxanes, NONOates, N-nitro derivatives, and many others. These preparations are reasonably classified as prodrugs since their pharmacological action is due to the preliminary metabolism and release of the true active compound, NO. It is a highly active compound produced in living organisms and plants by the enzymatic pathway from the amino acid arginine. NO causes the relaxation of vascular smooth muscles, prevents platelet aggregation, protects against pathogenic microorganisms, serves as a neurotransmitter, regulates programmed cell death and proliferation, and plays an important role in the reproductive system. The fundamental importance of NO is that it is a universal paracrine and endocrine regulator of body processes (Serezhenkov et al., 2020; Van de Wouw et al., 2021; Andrabi et al., 2023).

A biologically and chemically justified method of NO transport is its transfer in the form of complexes with iron and protein thiols or with the thiol-containing amino acid cysteine and the tripeptide glutathione. These are mainly mononitrosyl, dinitrosyl, and binuclear dinitrosyl iron complexes: MNIC, M-DNIC, or B-DNIC: (RS)4Fe(NO), (RS)2Fe(NO)2, and (RS)2Fe2(NO)4, respectively (Shekhter et al., 2007; Borodulin et al., 2013). Due to the chemical activity of NO, heme-containing proteins and iron-sulfur centers (ISCs) are the targets primarily exposed to NO. The body's defense system is activated by releasing NO to block metal-containing regulatory centers in case of ingesting pathogenic bacteria. At the same time, NO activates several bacterial transcription factors, OxyR, SoxR, NorR, FurR, DosR, RedE, and Wbl, as protection against nitrosative stress (Spiro, 2007).

The regulatory protein NsrR, a member of the superfamily of Rrf2 proteins, plays an important role in the cellular response to nitrosative stress in many pathogenic bacteria and suppresses the transcription of genes primarily involved in the detoxification of NO. It has been proven that NsrR reacts rapidly with one NO molecule to the [4Fe-4S] cluster to form iron-nitrosyl complexes associated with NsrR, leading to loss of protein binding to the DNA. The reaction with just one NO molecule to the NsrR dimer provides cancellation. This exceptional sensitivity of the binding of the NsrR protein to DNA justifies the importance of regulating NO detoxification systems to minimize damage to the bacterium caused by nitrosative stress (Crack and Le Brun, 2024).

In the early 1970s, the positive properties of free radicals were pointed out, and the involvement of reactive oxygen species (ROS) in a phenomenon called respiratory explosion in phagocytizing cells was studied. The imbalance between the production (for example, nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases)) and the elimination of ROS leads to a phenomenon known as oxidative stress. ROS can also be important secondary mediators in redox signaling pathways (Musaogullari et al., 2020; Harris and

DeNicola, 2020). Sulfhydryl groups of proteins (i.e., thiols) are capable of translating oxidant (ROS) signals into biochemical reactions, which leads to various post-translational, reversible modifications, including S-sulfenylation, S-nitrosylation, and S-glutathionylation. The most important of these modifications is S-glutathionylation, the reversible formation of mixed disulfides between protein-reactive thiols and the most common antioxidant, glutathione. In mammalian cells, glutathionylation acts as a regulatory mechanism and can protect proteins from irreversible oxidation. This fact is a fundamentally new view of the role of glutathione in the cell (Musaogullari and Chai, 2020). Low molecular weight (LMW) bacterial thiols are also involved in similar processes, playing a regulatory and protective role against ROS, heavy metals, toxins, and antibiotics (Gutiérrez-Fernández et al., 2024).

Thus, there is every reason for the practical use of NO donors (DNIC with thiol ligands) as regulators of the biochemical activity of animal and bacterial cells. It has been shown that the effective range of NO action extends from tens of nM (physiological regulation) to hundreds of μM when the cytotoxic effect is manifested. The study of the antiviral effect of NO donors has reached a fundamentally new level: the target of the NO action was the cysteine proteases of viruses responsible for entering the cell. The modification of proteases is reduced to the nitrosylation of cysteine and the formation of protein DNIC (Chen et al., 2020; Vanin et al., 2023; Pectol et al., 2020).

We used nitrosyl iron complexes with thiol ligands to treat wounds as an antihypertensive agent. Their composition is identical to the compounds that function in animals and humans as a transportable form of NO. An important advantage of DNIC is the possibility of varying the antioxidant component in its composition, i.e., the thiol compounds (cysteine, glutathione, and others). The modulating effect of DNIC is realized by the fact that the S-containing residue of the amino acid cysteine of proteins binds the Fe(NO)2 group released from DNIC (B-DNIC), which changes the activity of proteins. The inclusion of the Fe(NO)2 fragment into protein complexes ensures the stabilization of the free radical agent (NO) and its transport over considerable distances, i.e., both the autocrine and paracrine effects of NO are realized in living organisms (Borodulin et al., 2013). In vitro experiments have shown the formation of thiol disulfides, which are part of the DNIC structure (glutathione disulfide and cysteine) (Syrtsova et al., 2014). The latter circumstance suggests the synthesis of mixed disulfides in proteins. In this case, DNIC are involved in the post-translational modification of regulatory proteins in the cell through S-glutathionization.

Despite the abundance of pharmacological preparations for the treatment and prevention of metritis, endometritis, intrauterine infection (IUI) of cattle, and developing symptomatic infertility, significant progress has not been achieved. IUI in cattle is one of the most serious causes of decreased reproduction and productivity, which has led to the development of national programs and therapeutic strategies to control genital infection in many countries with developed dairy farming. Uterine disease in 40% of dairy cows can occur 7-10 days after calving (metritis). The frequency of metritis depends on many causes, but the maximum incidence sometimes reaches 36% and 50% (Markusfeld, 1987; Zwald et al., 2004). Subsequently, 15 to 20% of cattle have a clinical disease that persists for more than 3 weeks after calving (endometritis), and about 30% have chronic inflammation of the uterus without clinical signs of uterine disease (subclinical endometritis) (Sheldon et al., 2006; Gilbert et al., 2005; LeBlanc et al., 2002).

During pregnancy, the uterus is sterile, but after childbirth, the uterine lumen is almost always contaminated with a wide range of bacteria. However, the development of a clinical disease depends on the balance between host immunity and bacterial pathogenicity. *E. coli* and *A. pyogenes* are the most common bacteria isolated from the uterine cavity of cattle with uterine disease, as well as several anaerobic bacteria such as *Prevotella* spp., *F. necrophorum,* and *F. nucleatum* (LeBlanc et al., 2002; Sheldon et al., 2002; Williams et al., 2005; Griffin et al., 1974; Elliott et al., 1968; Bonnet et al., 1991). Infection of the uterus with *E. coli* seems to pave the way for subsequent infection with other bacteria or viruses (Wlliams et al., 2005; Dohmen et al., 2000; Donofrio et al., 2008). *A. pyogenes*, *F. necrophorum*, and *Prevotella* spp. act synergistically, increasing the likelihood and severity of uterine disease (Olson et al., 1984; Ruder et al., 1981). Bovine herpesvirus 4 (BoHV-4) is not the only virus consistently associated with uterine disease after calving in cattle (Thiry et al., 1992; Ackermann, 2006). Like other herpesviruses, BoHV-4 can cause latent infections in cattle, especially in macrophages (Donofrio and van Santen, 2001); viral infection is often recorded simultaneously with bacteria causing uterine disease (Monge et al., 2006; Frazier et al., 2001).

There may be a vicious circle consisting of bacterial endometritis leading to prostaglandin (PGE) secretion, after which PGE and bacterial lipopolysaccharide (LPS) stimulate viral replication, which causes further damage to endometrial tissue and inflammation (Sheldon et al., 2008).

Thus, cattle endometrial cells express elements of the LPS receptor complex and can respond to bacterial products, but this response depends on and is influenced by the level of reproductive hormones.

The development of bovine endometritis involves very complex signaling processes, including the detection of bacterial components by innate immune cells through toll-like receptors; the appearance of tumor necrosis factor-alpha (TNF-α) and other pro-inflammatory cytokines, for example, interleukins (IL); and also the mobilization of neutrophils followed by phagocytosis of the pathogens that entered the lumen of the uterus (Sheldon et al., 2008). The mechanism of

action of pro-inflammatory preparations, such as cytokines (for example, TNF-α, IL-1β, and IL-6), stimulates diapedesis and chemoattraction of neutrophils and monocytes and promotes increased phagocytosis. The production of proteins such as haptoglobin, acid glycoprotein, ceruloplasmin, or serum amyloid A is also stimulated (Tóthova et al., 2014). These regulatory chains have a direct and clear connection mediated by a signaling molecule such as NO (Cui et al., 2022).

Thus, to solve the accumulated problems in the treatment of IUI and inflammatory processes in the endometrium of cattle (cows and heifers), there is a real pharmacological tool in the form of NO donors (DNIC with thiol ligands), which allows not only to restore disrupted regulatory chains, replenishing the missing pool of NO but also directly have a bactericidal effect on pathogenic microflora and viruses. Currently, IUIs in cattle are associated with various causes: leptospirosis, viral diarrhea, infectious rhinotracheitis, and campylobacteriosis.

To evaluate the anti-inflammatory potential of nitric oxide donors prior to veterinary application, we employed a rat model of surgically induced endometriosis, which shares key inflammatory and angiogenic pathways with bovine endometritis. This study used an experimental model of endometriosis to evaluate the pathophysiological mechanisms and the effectiveness of new preparations. This model provides an opportunity to develop new treatment and prevention strategies, which is an important step in improving the quality of therapy for diseases such as IUI in cattle.

The study aimed to evaluate the effect of various NO donors: DNIC with thiol ligands (glutathione and thiosulfate), glutathione nitrosothiol, and dimethylguanidine (metformin) on surgically induced endometriosis in rats. A culture of the gram-positive bacteria *S. aureus* strain 209 P was used to evaluate the effective concentrations of DNIC with glutathione on pathogenic microflora. LMW nitrosyl iron complexes with thiol ligands were used as a pilot study of in vivo therapy in cows with diagnosed endometritis.

MATERIALS AND METHODS

***Synthesis of Nitrosyl Iron Complexes*:** Binuclear dinitrosyl iron complexes (B-DNIC) with glutathione were synthesized by treating an aqueous solution of ferrous sulfate (Fluka, Switzerland) with a glutathione solution (Sigma, USA) in a Thunberg vessel under NO atmosphere. The molar ratio of Fe^{2+} to glutathione was maintained at 1:2. Specifically, 0.5 ml of 5 mM $FeSO_4$ solution (pH 5.5) and 4.5 ml of 15 mM glutathione solution in HEPES buffer (pH 7.4) were placed in separate compartments of the vessel. After degassing and NO injection (100 mmHg), the vessel was shaken for 5 minutes to initiate reaction. NO was then removed under vacuum.

The resulting B-DNICs (formula: $(RS^-)_2[Fe(NO)_2]_2$) are EPR-silent. To generate EPR-active mononuclear complexes (M-DNICs), a 10-20-fold excess of glutathione or thiosulfate was added, and the pH was adjusted to 10-11. EPR spectra were recorded with characteristic g-values: $g\perp = 2.04$, $g\parallel = 2.014$. The distribution of complexes (MNIC : M-DNIC : B-DNIC) in the final preparation was approximately 0.5%, 17.5%, and 82%, respectively. Reactions were performed in triplicate to assess batch consistency; variation in EPR-integrated concentration across batches did not exceed ±5%.

***Bacteriological Assay Design*:** Antibacterial activity of DNIC-glutathione was tested against *Staphylococcus aureus* strain 209P. Bacterial suspensions (10^6 CFU/ml) were incubated in meat infusion broth (HiMedia) with DNIC concentrations of 3.6, 36, 180, and 360 µM. Control samples received saline only. All conditions were tested in three biological replicates.

After 14 hours of incubation at 37°C under aerobic conditions, colony-forming units (CFUs) were quantified by serial dilution and plating on meat infusion agar (MIA). Results were expressed as mean ± SD of CFUs per ml. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$ considered significant).

***Animal Experiments*:** Rat model and veterinary (cow) studies are described in detail in the Results section. However, briefly:

Rats (n=6 per group): Endometriosis was surgically induced, and animals received daily intraperitoneal injections of NO donors for 10 days. Cyst volumes were measured at necropsy.

Cows (n=4): Chronic endometritis was confirmed by clinical, microbiological, and cytological methods. Animals received daily intrauterine injections of DNIC-glutathione (180 µg/kg) for 5 days. Microbial load was assessed before and after treatment using standard culture techniques.

***Ethical Considerations*:** All animal procedures were approved by the institutional bioethics committee and conducted in accordance with relevant national and EU animal welfare regulations.

RESULTS

After incubation, 5 ml of suspension containing bacteria of each experimental group was sampled and diluted with saline solution with a phosphate buffer of 15 mM with pH=7.2. An aliquot of the suspension was transferred to Petri dishes with meat infusion agar (MIA) to count colonies and estimate the proportion of surviving bacteria. Gram-positive S.aureus bacteria with a concentration of 1 million/ml were incubated in a MIB medium with pH=7.2 at 37°C for 14 hours at four concentrations of DNIC (the ratio of iron: glutathione is 1:20): 360, 180, 36, and 3.6 µM. In experiments with concentrations of 360 and 180 µM of DNIC-glutathione, we did not observe bacterial growth after 14 hours. At concentrations of 36 and 3.6 µM, sediment was observed, and when seeded at MIA, growth and colony formation were detected. Table 1 shows

the quantitative measurement data. We observed colony formation for all control samples.

Table 1. The effect of nitrosyl complexes on colony formation of *S. aureus* bacteria strain 209 p.

	DNIC concentration, μM	Number of colonies, M ± m
Control	0	93.1 ± 37.2
Experiment 1	360	0
Experiment 2	180	0
Experiment 3	36	25.4 ± 13.2
Experiment 4	3.6	38.3 ± 19.4

25% of the bacteria remain viable when incubated with 36 μM DNIC-glutathione.

Our studies show that low concentrations of the NO donor (DNIC glutathione), 360-180 μM, cause cytotoxic effects on *S. aureus*. 100% cell death (complete absence of colonies) was detected in the case when the concentration of DNIC glutathione in the MIB medium was 360 and 180 μM. Seeding of *S. aureus* from control samples showed colony formation; their number was 93.1 ± 37.2.

The second stage was to study the effect of NO donors, DNIC with thiol ligands (glutathione), on surgically induced endometriosis in rats. The control group consisted of 16 animals. At autopsy, characteristic changes were observed in the developing endometriosis process. Fig. 1 shows a photograph of two transformed endometrioid implants in the shape of rounded cysts (tumors covered with a capsule).

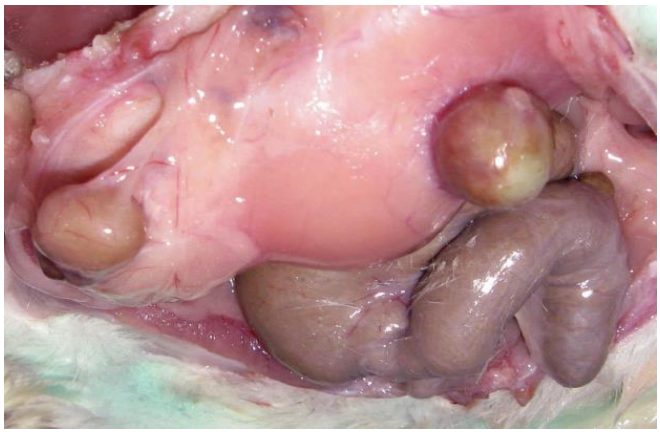

Figure. 1. Two endometrioid implants from a control animal.

In control samples of abdominal wall tissues, the presence of endometrioid glands, that is, ectopic endometrial cells, was recorded. There were signs of tissue degeneration (the formation of a fibrous pseudo capsule) and inflammation: the focus was filled with detritus and neutrophilic masses (Fig. 2).

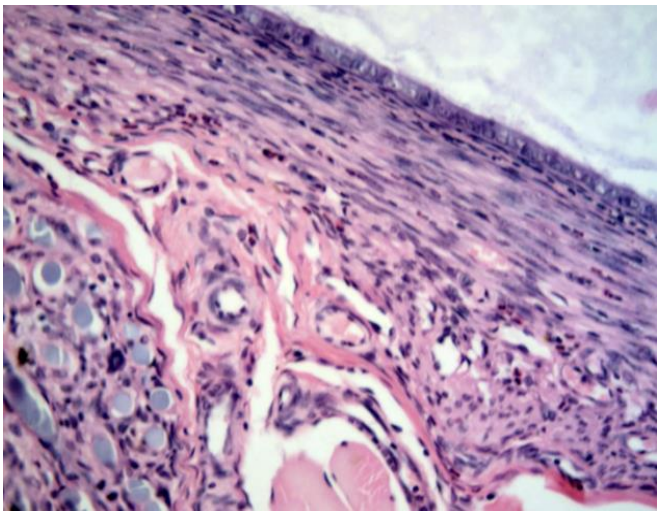

Figure 2. A fragment of the wall of an endometrioid cyst, with weakly basophilic contents in its lumen. Below, an endometrioid cyst adhered to the muscle and connective tissue of the anterior abdominal wall with a significant focus of pronounced dystrophic changes and necrosis of muscle cells and weak diffuse leukocyte infiltration. Hematoxylin and eosin staining (x400 scale).

According to the autopsy results of the control animals, the following conclusion can be drawn. Of the 26 sewn fragments of the fallopian tubes, all developed into endometrioid tumors (100% engraftment). At the time of opening, their size ranged from 78.9±44.3 to 87.9±31.5 mm^3 (Table 1) for one and two implants, respectively. The most informative characteristic of the sample, which has an abnormal distribution according to the results of statistical processing, is the median: 42.4-51.5 mm^3.

Table 2 shows the sizes of endometrioid foci after 10 days of therapy with NO donors. In the experiment, animals from groups 1 and 2 were injected intraperitoneally with 0.5 ml DNIC with glutathione at a dose of 8.0 μg/kg daily for 10 days. The animals of the third group received an aqueous solution of the DNIC complex with thiosulfate (1:20) at a dose of 4.7 μg/kg. For comparative study, NO donors with significantly different lifetimes were used. Thus, the binuclear DNIC with glutathione was the most stable NO donor in vitro, with a half-life of more than 30 days. DNIC with glutathione in a ratio of 1:10 retains the NO donor properties for about five days at 20°C. An even less stable NO donor is a thiosulfate ligand-based DNIC (1:10) that retains its properties for about 3-4 hours. The fourth preparation, dimethylguanidine (Sigma) (doses of 100 mg/kg), used as a NO donor, is being actively investigated in vitro and in vivo. First, dimethylguanidine has been used for more than 40 years

Table 2. Results of studies of endometrioid foci in the six groups of animals.

Groups of animals		1 implant (mm^3)(X± standard deviation (SD))	2 implant (mm^3)(X±SD)
Group 1, bi-nuclear DNIC with glutathione 1:2	Mean	11.5±12.5	15.1±13.3
	Median	6.5	12
Group 2, DNIC with glutathione 1:10	Mean	1.2±1.2	7.8±9.9
	Median	1	1.8
Group 3, DNIC 1:10 with thiosulfate	Mean	12.0±21.7	1.2±1.7
	Median	0.6	0.5
Group 4, dimethylguanidine (metformin)	Mean	13.2±15.4	16.1±18.5
	Median	6.8	8.9
Group 5, control animals	Mean	78.9±44.3	87.9±31.5
	Median	42.4	51.5

as an antidiabetic agent under various brand names (metformin, glucophage), lowering blood sugar levels, lowering blood pressure, and increasing insulin sensitivity. Second, it has found application in inhibiting polycystic ovarian diseases (Chen et al., 2022). According to our data, dimethylguanidine is almost completely transformed by releasing NO and forming DNIC.

The degree of inhibition, or rather regression, of endometrioid cysts (or foci) in the first experimental group (B-DNIC) was 6.3 times compared to the control group ($P < 0.05$) estimated by the value of the average total volume (TV) (or 5.0 times when comparing the medians). Metformin (group 4), which was close to B-DNIC in terms of the time of metabolism, caused cystic regression by 5.7 times by the value of the average TV ($P < 0.05$) or 6.0 times when comparing medians, which is consistent with the data of (Oner et al., 2011), where it was shown that metformin caused a decrease in cystic volume by 7.7 times at a dose of 100 mg/kg.

NO donors, which were less stable according to the results of in vitro experiments, showed similar values of the degree of regression of endometrioid cysts: the second group (DNIC with glutathione 1:10) showed the regression equaling 18.5 times when comparing the average TV ($P < 0.05$) and 33.6 times when comparing the medians. The maximum cystic regression rates were observed when exposed to DNIC with thiosulfate, equaling 15.0 times by the value of the average TV ($P < 0.05$) and 85 times when estimated by the median.

Previously, to explain the activation of apoptosis in Jurkat cell culture, the involvement of nitrosylated surface proteins in the treatment of DNIC with thiosulfate was assumed (Kleschyov et al., 2006). Another possible explanation is that rapidly decomposing DNIC with thiosulfate provides high concentrations of NO. The latter can block heme-containing proteins (for example, aromatase) or contribute to the formation of highly toxic peroxynitrite, resulting in cell death in endometrioid foci.

The results can be explained by blocking the activation pathways of transcription factor KB (NF-kB) (Arsura et al., 2003; von Knethen et al., 1999; Pirdel and Pirdel, 2014) through the formation of DNIC on proteins, for which exogenous DNIC (dimethylguanidine or a rapidly decaying thiosulfate complex) serve as a source of NO. There is every reason to believe that such a process can be implemented in a cell since local iron concentrations in endometrioid foci reach 100 μM (Pirdel and Pirdel, 2014; Kitajima et al., 2011).

The results we obtained in vitro and presented above looked reasonable for studying the antiendometritic effect of nitrosyl iron complexes in vivo in cows with diagnosed endometritis. A group of four animals was formed to study treating cow endometritis with an NO donor (DNIC) preparation. The body temperature in cows with endometritis was above the upper limit of the norm by an average of 0.5-1°C. Rectal examination showed that the uterus was enlarged, lowered into the abdominal cavity, and the uterine wall was flabby and

Table 3. Results of microbiological studies before the use of the preparation.

Cow	Microbiological media					
	2% MIA +1% glucose	2% MIA without glucose	MIB	Bulir's medium	Sabouraud's medium	Kitt-Tarozzi medium
1	13,600 microbial bodies	Continuous growth	Growth of *P. aeruginosa*, film, green sediment	*E. coli* 10^{-5}	*C. albicans*, Aspergilieus	Gas formation
2	18,300 microbial bodies	Continuous growth	Growth of *P. aeruginosa*, film, green sediment	*E. coli* 10^{-3}	Aspergillus, Mucor	Turbidity, gas formation
3	32,500 microbial bodies	The growth of individual milky-gray colonies on the entire surface	Negative	*E. coli* 10^{-2}	No growth	Growth in the upper part
4	14,600 microbial bodies	The growth of individual milky-gray colonies on the entire surface	Negative	*E. coli* 10^{-3}	Mucor	Negative

Table 4. Results of microbiological studies after the administration of the preparation.

Cow	Microbiological media					
	2% MIA +1% glucose	**2% MIA without glucose**	**MIB**	**Bulir's medium**	**Sabouraud's medium**	**Kitt-Tarozzi medium**
1	3,200 microbial bodies	Isolated cocci colonies	Negative	0.1	No growth	No growth
2	4,300 microbial bodies	Individual colonies, 7 units	Negative	Negative	No growth	No growth
3	6,200 microbial bodies	Individual colonies, 3 units	Turbidity without pigment	Negative	No growth	No growth
4	3,700 microbial bodies	Negative	Turbidity without pigment	Negative	Negative	Negative

edematous. A vaginal examination showed that the mucous membrane was edematous, bright red, with striated and mass/petechial hemorrhages. Serous-purulent and bloody discharge was observed in animals, which consisted of a mucous, cloudy, floccose-inclusion-containing exudate. The samples obtained during the work to assess the microbial composition were seeded on selective and differential diagnostic nutrient media: 2% MIA with 1% glucose, MIB, Bulir's medium, and Sabouraud's medium. The colonies were counted according to the State Standard (GOST). According to the results of the examination, the animals were diagnosed with chronic endometritis. Before treatment with DNIC, animals were treated with conventional preparations (Endometromag-K, Metricure) for a long time. Unfortunately, they did not receive positive results.

The animals were treated daily at 11 a.m. with an 80 ml intrauterine injection of an 8 mM aqueous solution of DNIC with glutathione (1:5) at a dose of 180 μg/kg. Before the administration of the preparation, samples were taken from the animals for microbiological analysis, and photo registration was performed. The results of the bacteriological examination before and after the use of the preparation are presented in Tables 3 and 4. Table 3 shows that initially, before the use of the preparation in the test group, the bacterial spectrum inhabiting the space from the vagina to the cervical canal was represented by a variety of microbial agents. These were cocci, *E. coli*, *P. aeruginosa*, and various fungi. The results of the microbial flora analysis after the preparation application are presented in Table 4. Cow 1 has a 4-fold decrease in the number of microbial bodies, cow 2 has a 4-fold decrease, cow 3 has a 5-fold decrease, and cow 4 has a 5-fold decrease. *No growth* of *P. aeruginosa* was observed in cows 1 and 2, and no growth of *E. coli* was detected in cows 1-3. The absence of fungal growth and anaerobic culture was observed in all cows.

In our opinion, the preparation used to treat endometritis has a cytotoxic effect due to the release of NO and through the direct interaction of the $Fe(NO)_2$ group with the thiol groups of microbial flora proteins. During the interaction of NO (nitrosyl iron complexes) with transcription factors DosR, OxyR, SoxR, NorR, FurR, and RedE (Spiro, 2007; Hausladen et al., 1996), cysteine residues included in the ISC structure can also be nitrosylated to form nitrosothiol (Zwald et al., 2004), which leads to disruption of their functions and ultimately to the death of microorganisms. The influence of exogenous LMW DNIC can also be realized at the level of enzymes involved in the metabolism of bacterial thiols, which are the most critical for microorganisms (Steenkamp, 2002).

Conclusions: A possible principle of operation of DNIC is the formation of complexes with signaling molecules, which leads to nitrosylation of target molecules. Modifying intracellular mediators can shift the balance of multiple signaling systems from cell proliferation to triggering cell apoptosis. The role of nitrosyl complexes is suggested by the diversity of the biological action of NO noted by many researchers. Thus, under normal conditions, it stimulates cell growth and survival, but in elevated concentrations and with an excess of free iron, its ability to stimulate proapoptotic signaling pathways is manifested. It is also necessary to consider the indirect effect on the growth of the endometrioid focus due to the ability of NO in elevated concentrations to inhibit angiogenesis (Groothuis, 2005). On the other hand, it promotes angiogenesis by activating cyclic guanosine monophosphate (cGMP)-dependent protein kinase (cGPK1) and stimulating the expression of vascular endothelial growth factor (VEGF) (Ziche, 2021). This is shown in healthy and tumor cells; NO synthase inhibitors are proposed as an antitumor treatment, and NO donors are used to stimulate angiogenesis.

Thus, the results obtained indicate a significant regression of endometrioid cysts during the 10-day treatment of rats with experimental endometriosis with DNIC used as NO donors. Binuclear and mononuclear DNIC with thiol ligands, DNIC with thiosulfate, and dimethylaniline, metabolized with the release of NO, activate apoptosis in fast-growing tissue.

DNIC therapy with glutathione in cattle with chronic endometritis has a pronounced bactericidal effect. The animals received intrauterine injections with a solution of DNIC-glutathione in a ratio of 1:5 (iron: glutathione) at a dose of 180 μg/kg and a volume of 80 ml once a day for 5 days. The use of the DNIC preparation with thiol ligands as a pharmacological agent requires further research.

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***Policy referred*:** This study aligns with veterinary public health strategies supporting sustainable livestock reproduction and antimicrobial resistance reduction, as outlined by the FAO and OIE guidelines.

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REFERENCES

Ackermann, M. (2006). Pathogenesis of gammaherpesvirus infections. *Veterinary Microbiology, 113*(3-4), 211-222. https://doi.org/10.1016/j.vetmic.2005.11.003

Andrabi, S. M., Sharma, N. S., Karan, A., Shahriar, S. S., Cordon, B., Ma, B., & Xie, J. (2023). Nitric oxide: Physiological functions, delivery, and biomedical applications. *Advanced Science, 10*(30), 2303259. https://doi.org/10.1002/advs.202303259

Arsura, M., Panta, G. R., Bilyeu, J. D., Cavin, L. G., Sovak, M. A., Oliver, A. A., Factor, V., Heuchel, R., Mercurio, F., Thorgeirsson, S. S., & Sonenshein, G. E. (2003). Transient activation of NF-κB through a TAK1/IKK kinase pathway by TGF-β1 inhibits AP-1/SMAD signaling and apoptosis: Implications in liver tumor formation. *Oncogene, 22*(3), 412-425. https://doi.org/10.1038/sj.onc.1206152

Artyushina, Z., Fedotov, S., Serezhenkov, V., Sidnev, N., Zherebtsov, I., Regan, R. G., & Muradyan, Z. (2024). Nitric oxide acts as an early diagnostic tool for intrauterine diseases in animal health. *Bangladesh Journal of Infectious Diseases, 11*(1), 52-58. https://doi.org/10.3329/bjid.v11i1.75566

Boni, R., & Gualandi, S. C. (2022). Relationship between oxidative stress and endometritis: Exploiting knowledge gained in mares and cows. *Animals, 12*(18), 2403. https://doi.org/10.3390/ani12182403

Bonnett, B. N., Martin, S. W., Gannon, V. P., Miller, R. B., & Etherington, W. G. (1991). Endometrial biopsy in Holstein-Friesian dairy cows. III. Bacteriological analysis and correlations with histological findings. *Canadian Journal of Veterinary Research, 55*(2), 168.

Borodulin, R. R., Kubrina, L. N., Serezhenkov, V. A., Burbaev, D. S., Mikoyan, V. D., & Vanin, A. F. (2013). Redox conversions of dinitrosyl iron complexes with natural thiol-containing ligands. *Nitric Oxide, 35*, 35-41. https://doi.org/10.1016/j.niox.2013.07.004

Chen, X., Leong, C. W., Flor, M., & Klebanov, B. B. (2020). Go figure! Multi-task transformer-based architecture for metaphor detection using idioms: ETS team in 2020 metaphor shared task. In *Proceedings of the Second Workshop on Figurative Language Processing* (pp. 235-243). https://doi.org/10.18653/v1/2020.figlang-1.32

Crack, J. C., & Le Brun, N. E. (2024). Binding of a single nitric oxide molecule is sufficient to disrupt DNA binding of the nitrosative stress regulator NsrR. *Chemical Science, 15*(45), 18920-18932. https://doi.org/10.1039/D4SC04618H

Cui, L., Li, Z., Xu, F., Tian, Y., Chen, T., Li, J., Guo, Y., & Lyu, Q. (2022). Antitumor effects of astaxanthin on esophageal squamous cell carcinoma by up-regulation of PPARγ. *Nutrition and Cancer, 74*(4), 1399-1410. https://doi.org/10.1080/01635581.2021.1952449

Dohmen, M. J. W., Joop, K., Sturk, A., Bols, P. E. J., & Lohuis, J. A. C. M. (2000). Relationship between intra-uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. *Theriogenology, 54*(7), 1019-1032. https://dx.doi.org/10.1016/S0093-691X(00)00410-6

Donofrio, G., & Van Santen, V. L. (2001). A bovine macrophage cell line supports bovine herpesvirus-4 persistent infection. *Journal of General Virology, 82*(5), 1181-1185. https://doi.org/10.1099/0022-1317-82-5-1181

Donofrio, G., Ravanetti, L., Cavirani, S., Herath, S., Capocefalo, A., & Sheldon, I. M. (2008). Bacterial infection of endometrial stromal cells influences bovine herpesvirus 4 immediate early gene activation: A new insight into bacterial and viral interaction for uterine disease. *Reproduction (Cambridge, England), 136*(3), 361.

Elliott, L., McMahon, K. J., Gier, H. T., & Marion, G. B. (1968). Uterus of the cow after parturition: bacterial content. *American Journal of Veterinary Research, 29*(1), 77-81.

Frazier, K., Pence, M., Mauel, M. J., Liggett, A., Hines, M. E., Sangster, L., Lehmkuhl, H. D., Miller, D., Styer, E., West, J., & Baldwin, C. A. (2001). Endometritis in postparturient cattle associated with bovine herpesvirus-4 infection: 15 cases. *Journal of Veterinary Diagnostic Investigation, 13*(6), 502-508. https://doi.org/10.1177/104063870101300608

Gilbert, R. O., Shin, S. T., Guard, C. L., Erb, H. N., & Frajblat, M. (2005). Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology, 64*(9), 1879-1888. https://doi.org/10.1016/j.theriogenology.2005.04.022

Griffin, J. F. T., Hartigan, P. J., & Nunn, W. R. (1974). Non-specific uterine infection and bovine fertility. I. Infection patterns and endometritis during the first seven weeks post-partum. *Theriogenology, 1*(3), 91-106. https://doi.org/10.1016/0093-691X(74)90052-1

Groothuis, P. A. (2005). Benefit transfer: A comparison of approaches. *Growth and Change, 36*(4), 551-564. https://doi.org/10.1111/j.1468-2257.2005.00294.x

Gutiérrez-Fernández, J., Hersleth, H. P., & Hammerstad, M. (2024). The crystal structure of mycothiol disulfide reductase (Mtr) provides mechanistic insight into the specific low-molecular-weight thiol reductase activity of Actinobacteria. *Acta Crystallographica Section D: Structural Biology, 80*(Pt 3),s181-193. https://doi.org/10.1107/S205979832400113X

Harris, I. S., & DeNicola, G. M. (2020, June 1). The complex interplay between antioxidants and ROS in cancer. *Trends in Cell Biology, 30*(6), 440-451. https://doi.org/10.1016/j.tcb.2020.03.002

Hausladen, A., Privalle, C. T., Keng, T., DeAngelo, J., & Stamler, J. S. (1996). Nitrosative stress: Activation of the transcription factor OxyR. *Cell, 86*(5), 719-729. https://doi.org/10.1016/S0092-8674(00)80147-6

Huerta-Saquero, A., Nefedova, E., Shkil, N. N., Pestryakov, A., & Bogdanchikova, N. (2025). AgNPs treatment reduces time recovery and increases bacterial sensitivity to antibiotics in cow´s purulent catarrhal endometritis. A translational study. *PLoS ONE, 20*(10), e0335305. https://doi.org/10.1371/journal.pone.0335305

Husnain, A., Arshad, U., Poindexter, M. B., Zimpel, R., Marinho, M. N., Perdomo, M. C., Fan, P., Jeong, K. C., Nelson, C. D., Sheldon, I. M., Bromfield, J. J., & Santos, J. E. P. (2023). Induced endometritis in early lactation compromises production and reproduction in dairy cows. *Journal of Dairy Science, 106*(6), 4198-4213. https://doi.org/10.3168/jds.2022-22846

Kasimanickam, R., Bhowmik, P., Kastelic, J., Ferreira, J., & Kasimanickam, V. (2025). From infection to infertility: Diagnostic, therapeutic, and molecular perspectives on postpartum metritis and endometritis in dairy cows. *Animals, 15*(19), 2841. https://doi.org/10.3390/ani15192841

Kitajima, M., Defrère, S., Dolmans, M. M., Colette, S., Squifflet, J., Van Langendonckt, A., & Donnez, J. (2011). Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. *Fertility and Sterility, 96*(3), 685-691. https://dx.doi.org/10.1016/j.fertnstert.2011.06.064

Kleschyov, A. L., Strand, S., Schmitt, S., Gottfried, D., Skatchkov, M., Sjakste, N., Daiber, A., Umansky, V., & Munzel, T. (2006). Dinitrosyl-iron triggers apoptosis in Jurkat cells despite overexpression of Bcl-2. *Free Radical Biology and Medicine, 40*(8), 1340-1348. https://doi.org/10.1016/j.freeradbiomed.2005.12.001

LeBlanc, S. J., Duffield, T. F., Leslie, K. E., Bateman, K. G., Keefe, G. P., Walton, J. S., & Johnson, W. H. (2002). Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *Journal of Dairy Science, 85*(9), 2223-2236. https://dx.doi.org/10.3168/jds.S0022-0302(02)74302-6

Markusfeld, O. (1987). Periparturient traits in seven high dairy herds: Incidence rates, association with parity, and interrelationships among traits. *Journal of Dairy Science, 70*(1), 158-166. https://dx.doi.org/10.3168/jds.S0022-0302(87)79990-1

Mason, R. P., & Josephy, P. D. (1985). An electron spin resonance investigation of the iron-catalyzed reaction of metronidazole with cysteine. *Journal of Inorganic Biochemistry, 24*(2), 161-165. https://dx.doi.org/10.1016/0162-0134(85)80008-8

Monge, A., Elvira, L., Gonzalez, J. V., Astiz, S., & Wellenberg, G. J. (2006). Bovine herpesvirus 4-associated postpartum metritis in a Spanish dairy herd. *Research in Veterinary Science*, *80*(1), 120-125. https://dx.doi.org/10.1016/j.rvsc.2005.04.001

Musaogullari, A., & Chai, Y. C. (2020). Redox regulation by protein S-glutathionylation: From molecular mechanisms to implications in health and disease. *International Journal of Molecular Sciences*, *21*(21), 8113. https://dx.doi.org/10.3390/ijms21218113

Musaogullari, A., Mandato, A., & Chai, Y. C. (2020). Role of glutathione depletion and reactive oxygen species generation on caspase-3 activation: A study with the kinase inhibitor staurosporine. *Frontiers in Physiology*, *11*, 998. https://dx.doi.org/10.3389/fphys.2020.00998

Nyabinwa, P., Kashongwe, O. B., Hirwa, C. D., & Bebe, B. O. (2020). Perception of farmers about endometritis prevention and control measures for zero-grazed dairy cows on smallholder farms in Rwanda. *BMC Veterinary Research, 16*(1), 175. https://doi.org/10.1186/s12917-020-02368-6

Oladejo, A. O., Li, Y., Wu, X., Imam, B. H., Yang, J., Ma, X., Yan, Z., & Wang, S. (2021). Modulation of bovine endometrial cell receptors and signaling pathways as a nanotherapeutic exploration against dairy cow postpartum endometritis. *Animals*, *11*(6), 1516. https://doi.org/10.3390/ani11061516

Olson, J. D., Ball, L., Mortimer, R. G., Farin, P. W., Adney, W. S., & Huffman, E. M. (1984). Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. *American Journal of Veterinary Research, 45*(11), 2251-2255.

Oner, G., Ozcelik, B., Ozgun, M. T., & Ozturk, F. (2011). The effects of metformin and letrozole on endometrium and ovary in a rat model. *Gynecological Endocrinology, 27*(12), 1084-1086. https://dx.doi.org/10.3109/09513590.2011.589928

Paiano, R., Moreno, L., Gomes, V., Parra, B., Barbosa, Sato, M., Bonilla, J., Pugliesi, G., Baruselli, P., & Moreno, A. (2022). Assessment of the main pathogens associated with clinical and subclinical endometritis in cows by culture and MALDI-TOF mass spectrometry identification. *Journal of Dairy Science*, *105*(4), 3367-3376. https://doi.org/10.3168/jds.2021-20642

Pascottini, O. B., LeBlanc, S. J., Gnemi, G., Leroy, J. L. M. R., & Opsomer, G. (2023). Genesis of clinical and subclinical endometritis in dairy cows. *Reproduction, 166*(2), R15-R24. https://doi.org/10.1530/rep-22-0452

Pectol, D. C., Khan, S., Elsabahy, M., Wooley, K. L., Lim, S. M., & Darensbourg, M. Y. (2020). Effects of glutathione and histidine on NO release from a dimeric dinitrosyl iron complex (DNIC). *Inorganic Chemistry*, *59*(23), 16998-17008. https://doi.org/10.1021/acs.inorgchem.0c02196

Pirdel, L., & Pirdel, M. (2014). Role of iron overload-induced macrophage apoptosis in the pathogenesis of peritoneal endometriosis. *Reproduction, 147*(6), R199-R207. https://doi.org/10.1530/REP-13-0552

Ruder, C. A., Sasser, R. G., Williams, R. J., Ely, J. K., Bull, R. C., & Butler, J. E. (1981). Uterine infections in the postpartum cow: II. Possible synergistic effect of Fusobacterium necrophorum and Corynebacterium pyogenes. *Theriogenology*, *15*(6), 573-580. https://dx.doi.org/10.1016/0093-691X(81)90060-1

Serezhenkov, V. A., Tkachev, N. A., Artyushina, Z. S., Kuznetsova, M. I., Kovac, M., & Vanin, A. F. (2020). Reduced nitric oxide bioavailability in horses with colic: Evaluation by ESR spectroscopy. *Biophysics*, *65*, 869-875. https://dx.doi.org/10.1134/S0006350920050176

Shekhter, A. B., Rudenko, T. G., Serezhenkov, V. A., & Vanin, A. F. (2007). Dinitrosyl iron complexes with thiol ligands promote skin wound healing in animals. *Biophysics*, *52*, 515-520. https://dx.doi.org/10.1134/S0006350907050177

Sheldon, I. M., Cronin, J., Goetze, L., Donofrio, G., & Schuberth, H. J. (2009). Defining postpartum uterine disease in cattle. *Theriogenology, 71*(1), 155-162. https://dx.doi.org/10.1016/j.theriogenology.2008.09.023

Sheldon, I. M., Lewis, G. S., LeBlanc, S., & Gilbert, R. O. (2006). Defining postpartum uterine disease in cattle. *Theriogenology*, *65*(8), 1516-1530. https://dx.doi.org/10.1016/j.theriogenology.2005.08.021

Sheldon, I. M., Owens, S. E., Turner, M. L., & Cronin, J. (2014). The postpartum uterus: Insights from the cow. *Reproduction, Fertility and Development*, *26*(1), 1-16. https://dx.doi.org/10.1071/RD13218

Soe, S., Sato, H., & Takahashi, K. (2022). Therapeutic strategies for endometritis in postpartum dairy cows. *Veterinary Sciences*, *9*(3), 120. https://doi.org/10.3390/vetsci9030120

Thompson, J., Bromfield, J., & Sheldon, I. (2012). Toll-like receptor and NOD-like receptor signaling in bovine endometritis. *Reproduction, 143*(6), 715-727. https://doi.org/10.1530/REP-11-0472

Vasquez, A., Gonzalez, L., & Guillemette, C. (2023). Redox regulation in bovine endometrial epithelial cells: Implications for postpartum uterine health. *Antioxidants*, *12*(11), 2210. https://doi.org/10.3390/antiox12112210

Vanin, A. F., Burbaev, D. S., Serezhenkov, V. A., & Sidorov, A. V. (2008). Biochemistry and pharmacology of dinitrosyl iron complexes with thiol-containing ligands. *Nitric Oxide*, *19*(1), 50-58. https://doi.org/10.1016/j.niox.2008.04.001

Williams, E. J., Fischer, D. P., Pfeiffer, D. U., England, G. C., Noakes, D. E., & Dobson, H. (2005). Clinical evaluation of endometritis in dairy cows. *Veterinary Record, 157*(20), 625-632. https://doi.org/10.1136/vr.157.20.625

Wolf, E., Braun, B., & Holtz, W. (2004). Endometrial prostaglandins and postpartum uterine involution in dairy cows. *Reproduction, 127*(4), 533-544. https://doi.org/10.1530/rep.1.00173

Wu, S., Liu, J., Sun, X., Li, J., Xu, L., & Zhang, D. (2021). Sources of heavy metal contamination in agricultural soils and associated health risks. *Science of The Total Environment, 776*, 145938. https://doi.org/10.1016/j.scitotenv.2021.145938

Zwolak, I., Sarzynska, M., & Składanowski, M. (2019). Sources of heavy metal contamination in agriculture. *Environmental Monitoring and Assessment, 191*(5), 310. https://doi.org/10.1007/s10661-019-7433-7